cells and that they localize to specific compartments. Based upon our observations, we hypothesize that crowders can influence stem cell differentiation by influencing molecular kinetics.

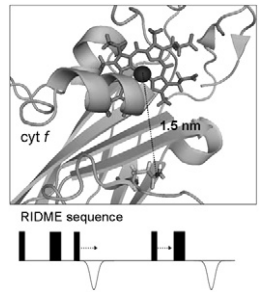
772-Pos Board B572
Volume and Morphological Changes in Red Blood Cells with Pressure Probed by Optical Imaging In-Situ
Silki Arora, Sang Hoon Park, Jennifer Mauser, Debopam Chakrabarti, Alfons Schulte.
Functional properties of living cells depend on the thermodynamic variables temperature and pressure. A unique tool to investigate volume effects on structure and metabolism of the cell is pressure perturbation. We employ a setup that allows visualizing individual live cells at variable pressure in real time. We present measurements of volume changes in red blood cells (RBC) over the pressure range from 0.1 to 200 MPa. Up to a pressure of 35 MPa the size of a healthy erythrocyte remains constant. Over the pressure range from 35 MPa to 200 MPa the lateral diameter decreases linearly and reversibly with a slope of 0.015 micron / MPa, while there are no significant alterations in shape. The RBC deformability is discussed in terms of the cell membrane elasticity and effects of the cytoskeletal network. Our experiments are extended to RBCs infected with the malaria parasite *Plasmodium falciparum*. Here, we observe clear differences in the deformability with pressure and between the compression and decompression curves.

773-Pos Board B573
In Vivo Imaging of Tumor Cell Migration
Christian Weis, Andreas Hess, Tim St. Pierre, Ben Fabry.
The process of metastasis formation involves the migration and 3-D invasion of tumor cells from a primary tumor to distant sites. We propose that the dynamics of the migration and invasion process of magnetically labeled tumor cells can be monitored in animal models over prolonged time periods using magnetic resonance imaging (MRI). Human breast carcinoma cells (MB-MDA-231) were labeled with superparamagnetic Fe_2O_3 iron oxide nanoparticles coated with poly-L-lysine. The particles are readily taken up by cancer cells and stored in intracellular clusters. During cell division, the nanoparticle clusters are divided and split unevenly between daughter cells (mean partitioning fraction 0.85 to 0.15). Nanoparticles are non-toxic, are not degraded by the cell and remain stable for at least 3 weeks. In vitro collagen gel assays show no differences in contractile properties and invasion behavior of magnetically labeled vs. non-labeled tumor cells. MRI of cells suspended in agarose gave a detection limit of the spin-spin-relaxation-rate above the agar background of approximately 70 cells per 1 mm^3. The minimal detection volume of tumor cells in agarose was 25 µl. Detection limit and minimal volume were verified by injecting labeled cancer cells in mice. Spin-spin-relaxation-weighted (T2-weighted) and susceptibility-weighted images show a rapid relaxation behavior and pronounced phase shifts in the vicinity of the injection area compared to control scans. These studies demonstrate the feasibility of the method for long-term observation of cancer cell migration in vivo with MRI.

774-Pos Board B574
Effect of Formalin Fixation on CARS Microscopy of Neural Tissue
Yeon Ho Kim, **Timothy J. O'Leary**, Jeffrey T. Mason.
The cognitive impairments associated with blast-induced mild traumatic brain injury (bmTBI) suggest that exposure to blast may disrupt the connectivity of the fiber tracts that form the neural network of the brain. Coherent anti-Stokes Raman scattering (CARS) microscopy is ideally suited to observe blast-induced structural changes in the myelinated axons of the neural white matter. The sensitivity of the CARS technique to the CH_2 stretching vibrations of the myelin sheath of the axons allows for the label-free imaging of fiber tracts at high spatial resolution with large depth penetration. To date, most CARS studies have been performed on living *in vivo* and *ex vivo* tissues. However, studies of the structural changes associated with bmTBI will require the ability to examine *post mortem* tissue. In this study, we report our findings on the use of CARS microscopy to observe myelin fibers in formalin-fixed mouse and porcine brain tissue. Neural structures including the cerebrum, spinal cord, corpus callosum, and hippocampus were examined. Our findings demonstrate that CARS microscopy can be used to determine fiber orientation and continuity, fiber area percentage, myelin density, and the g-ratio of individual myelin axons in neural tissue fixed in formalin for up to 3 months. In conclusion, we demonstrate that the spectroscopic and morphological artifacts produced by formalin fixation do not interfere with the ability of CARS microscopy to observe and characterize the structure of fiber tracts in formalin-fixed neural tissue.

EPR Spectroscopy

775-Pos Board B575
Distances Between Paramagnetic Metal Centers and Spin Labels in Proteins by Pulsed EPR: The RIDME Method As a New Tool
Sergey Milikisyants, Francesco Scarpelli, Michela Finiguerra, Marcellus Ubbink, **Martina Huber**.



Structure determination in biological systems by electron paramagnetic resonance (EPR) is becoming increasingly popular. Distances in the nm range between spin labels in proteins yield structure restraints[1]. Transition metal-ion centers abound in proteins, but their potential as markers for distance determination is limited by their large g-anisotropies and fast relaxation times.
For many of these centers, the known pulse sequences for e.g. DEER or PELDOR cannot be applied because of excitation bandwidth limitations. The RIDME method[2] circumvents this problem by making use of the spin-lattice relaxation (T_1)-induced spin-flip of the transition-metal ion. Designed to measure distance between such a fast relaxing metal center and a radical, it suffers from a dead time problem. This disadvantage can be avoided by the five-pulse RIDME (5p-RIDME) sequence. An Fe(III)-spin label distance in ths protein cytochrome *f* is determined.[3]
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776-Pos Board B576
The Global Analysis of DEER Data
Eric J. Hustedt.
Previously, global analysis has been successfully used to analyze both continuous wave and saturation transfer EPR data[1-3]. In this work, algorithms have been developed for the global analysis of DEER data. Applications include the analysis of DEER data collected at multiple frequencies or multiple timescales. Analysis of DEER data from the soluble protein CDB3 (MW ≈ 90 kD) has shown that the background DEER signal is not well-fit by an exponential decay due to the large size of the CDB3 dimer. As a result, background correction with an exponential decay prior to analysis results in a poor fit to the data. An algorithm has been developed which explicitly fits the background signal with the radius of the molecule (assuming it is spherical) and the spin concentration as parameters. Using this approach, excellent fits to DEER data can be obtained without prior background correction. Also, DEER data can be globally analyzed to determine changes in the relative populations of components of the distance distribution as a function of experimental conditions. For example, DEER has been previously used to study the structural effects of a proline to arginine mutation at residue 327 of CDB3. Intradimer distances in spin-labelled wild type CDB3 can be fit using a single component distance distribution[4]. The same measurements on P327R CDB3 indicate the mutation induces a second more disordered component in the distance distribution[5]. The global analysis of DEER data collected for multiple spin-labelling sites in both the WT and P327R background is being used to further test this two-component model.
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777-Pos Board B577
Nitroxide Spin Label Side Chain Dynamics of Solvent Exposed Sites on Membrane Proteins
Brett M. Kroncke, Justin Kim, Linda M. Columbus.
Understanding the structure and functional dynamics of membrane proteins in their native, hydrophobic environment is key to understanding how proteins function. EPR spectroscopy in combination with site directed spin labeling (SDSL) has the potential to quantify structure and dynamics of proteins of arbitrary weight in their native lipid environment. Several studies have elucidated the structural origins of CW EPR lineshapes of water-soluble proteins; however, CW EPR spectra of nitroxide spin labeled proteins in a detergent/lipid environment have characteristic differences from their water-soluble counterparts. Membrane protein spectra are generally broader and frequently